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Project Summary

Persistence of Pathogens in Lagoon-Stored Sludge

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The project objective was to investigate pathogen inactivation in lagoon-stored municipal sludges. The in-field lagoons were located in Louisiana (New Orleans) and in Texas (Port Aransas), both semitropical areas of the United States. Each lagoon was filled with 7.56 m^3 of anaerobically digested sludge to which a spike containing a mixture of *Salmonella livingstone*, poliovirus Type 1, and *Ascaris suum* eggs was added. The municipal sludge placed in each lagoon was from the respective local area. The field and laboratory data demonstrated that 15 mo of storage was required for pathogen inactivation to meet the U.S. Environmental Protection Agency's (EPA) Process to Further Reduce Pathogens (PFRP) criteria for lagoon-stored sludges in a semitropical climate. In this study, viable *Ascaris* eggs were inactivated in 15 mo in the New Orleans lagoon where the temperature averaged about 25°C over a 5 mo period. Although a similar temperature was observed for the Texas (Port Aransas) lagoon, all *Ascaris* eggs were dead after 12 mo of storage, probably because of petroleum organics in the Texas sludge. *Salmonella livingstone* was inactivated in 4 to 6 mo in both lagoons at a log-reduction rate of 1.2 and 1.6 log Most Probable Number (MPN)/mo/100 mL in New Orleans and Port Aransas sediments, respectively. Total coliforms and fecal coliforms declined 2 to 6 logs within 12 mo. Little, if any, die-off of fecal streptococci, either on a volume or a gram dry weight basis, was noted in either lagoon. An increase of total coliforms was observed in both lagoons after 10 mo. Poliovirus Type 1 was inactivated within 12 mo at rates ranging from 0.01 to 0.02 log PFU/mo/100 mL in the sediments of both lagoons.

This Project Summary was developed by EPA's Risk Reduction Engineering Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering form at the back).

Introduction

Sludges from municipal wastewater treatment plants often contain pathogens that are hazardous to humans and domestic animals. Partly because of this, Congress passed the Clean Water Act of 1977 (P.L. 95-217). This act led to the establishment of criteria for the control of pathogens related to the land application of sewage sludge (40 CFR 257). These criteria specify the minimum level of treatment needed before municipal wastewater sludges can be applied to land. In addition, EPA designated three categories of municipal sludges destined for agricultural use: "Not stabilized" (raw sludge), "Processes to Significantly Reduce Pathogens" (PSRP), and "Processes to Further Reduce Pathogens" (PFRP).

The criterion for a PSRP is that the process reduces pathogenic viruses by 1 log or 90% and indicator bacteria (fecal and total coliforms) by 2 logs or 99%. For a PFRP process, pathogens are to be reduced below detectable limits; i.e., 1 PFU for viruses, 3 MPN for pathogenic bacteria, and 1 viable helminth egg per 100 mL of sludge. In November 1986, an

EPA task force was established to review, on a case-by-case basis, processes not listed in the *Federal Register* that nevertheless attain the required pathogen reductions for either classification. Lagoon storage, which is the subject of this investigation, is one such process.

Lagoon storage of domestic waste sludges as a means of inactivating pathogens has received little attention. Results from a recent EPA-funded laboratory study indicated that when small amounts of sludge containing parasite eggs were stored at 25°C, the eggs were destroyed after 10 to 16 mo but that when sludge was stored at 4°C, some *Toxocara canis* and *Ascaris suum* eggs were still viable after 25 mo. These results suggested that storing sludges in lagoons in warm climates might be an effective way of inactivating parasite eggs and other pathogens and, in part, led to the initiation of this project.

The overall objective of this study was to evaluate the effectiveness of sludge storage in lagoons as a method of inactivating pathogens. A specific objective was to determine whether the storage of selected anaerobic sludges in lagoons in areas where the mean ambient temperatures approach 25°C could be an effective way of inactivating selected parasite eggs (*Ascaris suum*), *Salmonella*, and enteroviruses (poliovirus Type 1). An additional objective was to detect changes in sludge characteristics that could be correlated with pathogen inactivation.

Procedure

A field lagoon was placed at the Tulane University F. Edward Hebert Research Center in Belle Chasse, LA, and at the Nueces County Sewage Treatment Plant No. 5 in Port Aransas, TX. These locations were chosen for the climatic and environmental conditions associated with each. The size of each sludge lagoon was about 10.3 m^3, and each was filled with 7.56 m^3 (2,000 gal) of anaerobically digested municipal sludge from New Orleans, LA, and Corpus Christi, TX, respectively. These sludges were spiked with *Salmonella livingstone*, *Ascaris suum* eggs, and poliovirus Type 1. The parasites were run through bench scale digesters so that the spike would better simulate field conditions. Samples were taken and analyzed for bacteria and viruses at 24 hr, 1, 2, 4, 6, 9, 15, and 24 mo; parasite samples were taken and analyzed every 3 mo. At each sampling period, abiotic parameters were analyzed for oxygen, temperature, oxidation-reduction potential (ORP), pH, chemical oxygen demand (COD), solids (total and volatile) (VSS), ammonia, total Kjeldahl nitrogen, alkalinity, hardness, nitrite, and nitrate. Bottom and top samples were taken in five different locations. All biotic and abiotic analyses had the appropriate quality control and assurance testing. In addition, various knowns were also tested to ensure the precision and accuracy of the analytical test. To verify the lagoon results in regard to *Ascaris* eggs, 20 L of anaerobically digested municipal sludge from the New Orleans and 20 L from the Port Aransas area were each spiked with *Ascaris suum* eggs and stored in large plastic columns in the field in the New Orleans area. Samples were taken initially and every 3 mo thereafter.

Since there were no data concerning the rate of settling for *Ascaris suum* eggs, sludge settling tests were conducted in a 1.8 m settling column with sampling ports 0.3 apart. At 3, 6, 12, and 24 hr, 1 wk and 1, 2, and 3 mo, samples for *Ascaris suum* eggs were taken and suspended solids were analyzed .

All the sludge storage data were analyzed for statistical significance of pathogen die-off rate as a function of such abiotic factors as temperature, VSS, COD, pH, ORP, etc.

Results and Discussion

Bacterial Survival

The data pertaining to the inactivation of indicator bacteria indicated that the survival patterns for the coliforms and fecal streptococci were of the order of 2 to 4 magnitudes of inactivation over 1 yr. Although declines in indicator bacteria (log MPN/gm suspended solids) were preceded by a significant decrease in *Salmonella* during the first 3 mo of lagoon storage, the correlation of decreases in indicator bacteria with decreases in *Salmonella* appeared to be nebulous. This becomes more obvious when the increases in indicator organism concentrations after the first year of storage are examined – increases that continued through the second year as a possible result of outside contamination. The importance of *Salmonella* being reduced by more than 5 logs means that lagoon storage under certain semitropical conditions meets the criteria for PFRP in relation to inactivation of pathogenic bacteria (Figure 1).

Virus Survival

As can be seen in Figure 2, the analyses of thermal control samples (1 mL vials of poliovirus in their original medium) from both the New Orleans the Port Aransas lagoons revealed ne identical decreases in virus concen tions after 180 days of storage in lagoons' sediment fraction. Although thermal control concentrations decrea by approximately 2 logs, concentrat reductions approaching 6 logs w observed in the liquid and sedim fraction samples collected over the sa time period.

For both lagoons, poliovirus Type survival was significantly greater in sediment than the liquid fractions. Initi it was suspected that survival in the liq phase was greater in the New Orle than in the Port Aransas lagoon, but weight conversions showed that the s vival pattern differences could attributed to differences in initial conc trations following spiking. As can be se in Figure 2, which compares dry wei titration results for both the New Orlea and Port Aransas lagoon virus samples adjustments are made for the differenc in initial spiking concentrations (appr imately 1 log), the inactivation curv over storage time are nearly identic Thus, the titration differences could attributed to one of two facts: (1) t initially lower virus concentrations in t Port Aransas lagoon could have be from poor mixing and sampling, or (2) t petroleum constituents in the Port Ara sas lagoon sludges reacted immediate to inactivate the spiked virus durir mixing, but after mixing and lagoo storage, the chemical constituents ha little or no effect on virus survival.

Parasite Survival

Lagoon-Stored Sludge

The results of parasite analyses samples from the two lagoons are show in Figures 3 and 4 (Sludge Lagoo where the percent inactivation of *Ascar* eggs over time is shown. In the Ne Orleans lagoon (Figure 3), a die-off of th eggs began to occur after 3 mo and by mo 33% of the viable eggs had bee inactivated. By 9.3 mo, 74% of the egg had been inactivated, and by 12.2 m 98% of the eggs had been inactivate No viable eggs were recovered subse quently.

In the case of the Port Aransas lagoor the die-off of *Ascaris* eggs occurre much more rapidly. After 3 mo, 89% the viable eggs in the original spike ha been inactivated, and by 6.5 mo, near total inactivation (99.9%) had occurre The difference in the die-off of eggs i

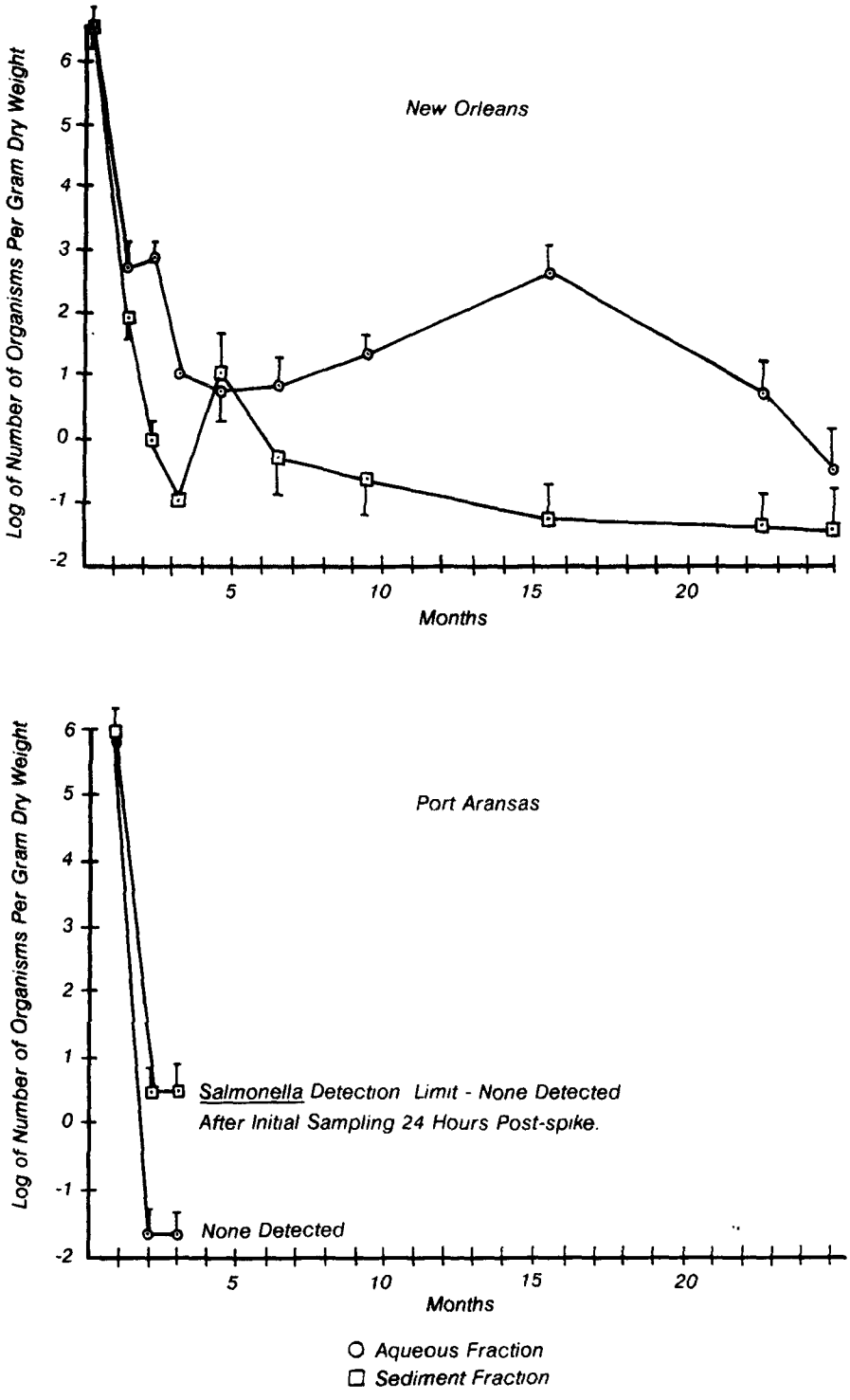

Figure 1. *Graph of* Salmonella *organisms per gram dry weight versus time in months for New Orleans and Port Aransas sludge lagoons.*

the two lagoons was significant ($p = 0.004$).

Small PVC tubes were placed in each lagoon and filled with sludge spiked with a higher concentration of *Ascaris* eggs so that the die-off of eggs could be monitored more accurately. In the sludge in the tube placed in the New Orleans lagoon, the eggs died off at about the same rate as the eggs in the lagoon sludge (Figure 3). The die-off in the tube placed in the Port Aransas lagoon was also similar to that in the sludge in the same lagoon (Figure 4). The *Ascaris* eggs in distilled water in vials placed in each lagoon died off within about 6 mo (Figure 5).

Sludge Stored in Large Columns

This part of the project was initiated to verify certain results obtained in the study of the survival of *Ascaris* eggs in sludge stored in the lagoons: the more rapid die-off of eggs in the Port Aransas lagoon sludge as compared with that in the New Orleans sludge, and the rapid die-off of eggs in the distilled water controls in both lagoons. In addition, the *Ascaris* eggs used in spiking the sludge in each lagoon had a relatively low level of viability, 13.7%, and it was desirable to determine if another batch of *Ascaris* eggs with a higher initial rate of viability could survive longer in stored sludge.

The inactivation of *Ascaris* eggs in sludge stored in the two large plastic columns is shown in Figures 3 and 4. The viability of the *Ascaris* eggs in the digested sludge used to spike these columns was 90.6%. In the column with New Orleans sludge, significant die-off did not occur until after 9 mo of storage. At 9 mo, only 11% of the viable eggs in the initial samples had been inactivated, but after that, the die-off was more rapid. At 12 mo, 76% of the eggs had been inactivated, and complete inactivation was observed at 15 2 mo

In the case of the eggs in the sludge of the Port Aransas column, i.e., sludge from Corpus Christi, TX, the rate of inactivation of *Ascaris* eggs was less than that observed in the New Orleans column. In the Port Aransas column, the inactivation of the eggs occurred at a fairly steady rate with 7% inactivation observed at 1 mo, 23% at 3 3 mo, 54% at 6.4 mo, 90% at 9 mo, and nearly complete inactivation (99.9%) at 12 mo Although the time it took for complete inactivation of eggs to occur in the sludge in the two columns was not statistically different, the inactivation that had occurred in the Port Aransas column at 6

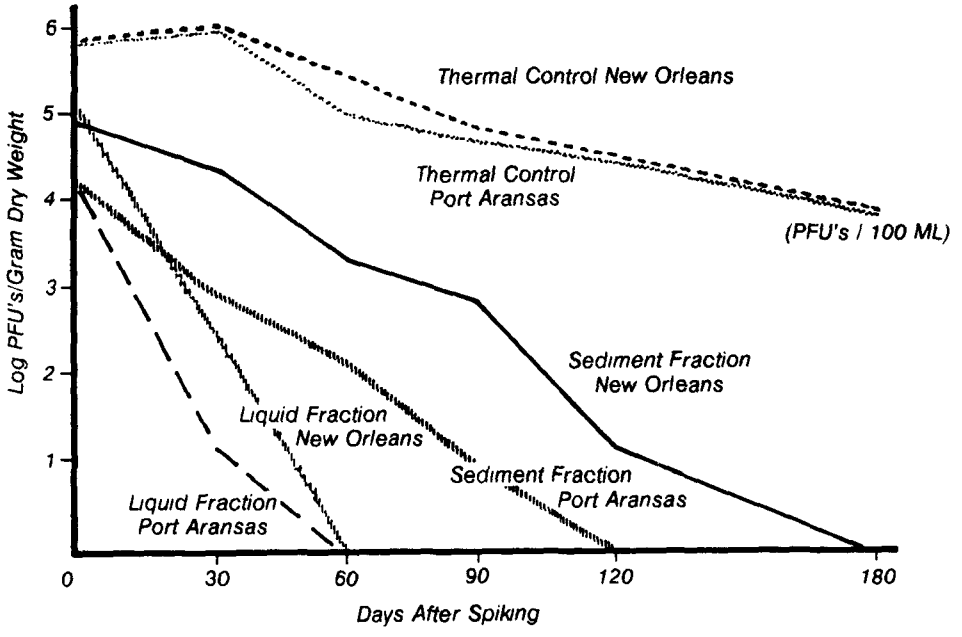

Figure 2. *Composite of Poliovirus survival New Orleans and Port Aransas lagoons.*

and 9 mo was significantly greater than the inactivation that had occurred at these times in the New Orleans column ($p < 0.05$).

Eggs in the distilled water controls placed in the two columns died at similar rates, although there was considerable fluctuation in the viability of the eggs from individual vials examined between 3 and 12 mo of storage. In the vials examined from each column at 12 mo, nearly all of the eggs had died (96% and 98%), and at 15.2 mo no viable eggs were observed in vials taken from the two columns. The inactivation of eggs in these distilled water controls was not significantly different from the inactivation of eggs in the column with the New Orleans sludge.

The storage of *Ascaris* eggs in sludges in lagoons and in large columns produced several interesting results. First of all, the eggs survived for a significantly shorter time in the Port Aransas lagoon than in the New Orleans lagoon. This difference in the survival of eggs in the two lagoons was probably due to the presence of petroleum by-products in the sludge in the Port Aransas lagoon, which affected the viability of the eggs. Since the temperatures observed in the two lagoons varied little from each other, temperature was unlikely to have caused the observed difference.

While the *Ascaris* eggs stored in Corpus Christi sludge in the column survived longer than they did in the sludge stored in the Port Aransas lagoon, they were inactivated at a more rapid rate than were the eggs in the New Orleans sludge column. This again was attributed to the presence of petroleum by-products in the Texas sludge. The *Ascaris* eggs in the New Orleans sludge column survived longer and had a later onset of inactivation than did the eggs in the New Orleans sludge lagoon. These reactions are partly attributable to the 90.6% rate of egg viability in the column spike as compared with the 13.7% for the lagoon spike.

The results of the settling experiment showed that when the anaerobic sludge containing *Ascaris* eggs was allowed to settle under quiescent conditions, some eggs still remained in the upper 30 cm of the sludge for at least 7 days. At 24 hr, only approximately 20% of the original number of eggs remained in the upper one-half (1 meter) of the settled sludge and, after 1 wk, less than 1% of the eggs were in the upper one-half of the sludge. After 1 mo, all of the eggs were found in the bottom 1 m of the sludge column. The solids in the sludge settled at ab the same rates as the *Ascaris* eggs.

When these results are compared v those in previous studies, it would app that *Ascaris* eggs settle more slowly in anaerobic sludge than in raw sewa This is to be expected since anaero sludge is thicker.

For the New Orleans municipal slud changes in abiotic parameters th correlated with *Ascaris* egg inactivati included only volatile solids. Fe streptococcus inactivation correlated w the fluctuation of volatile solids a temperature. The inactivation of oth indicator organisms and *Salmonella* w not observed to correlate with any abio changes. For the Corpus Christi mu cipal sludge, no abiotic parameters c related with *Ascaris* egg inactivatic Poliovirus inactivation correlated wi changes in pH, volatile solids, and to solids. Fecal streptococcus inactivati correlated only with conductivity. Th abiotic variables could not be used indicators of inactivation, but the abiotic parameters were related to th mechanism by which the die-o occurred.

Recommendations

Study results indicate that lagoc storage of municipal sludges und certain conditions in semitropical climate can be used to inactivate pathogens. was also observed that *Ascaris* egg were far more resistant to inactivatic than were bacteria and viruses. Based c these findings the following r recommended:

1) Initiate drying bed studies, with th use of raw sludge and aerobically an anaerobically treated sludges, t determine the exact stabilizatio conditions needed for producin sludges that meet PFRP criteria.
2) Investigate the applicability of usin combined treatment processe (digestion followed by lagoon and/c drying bed storage) to inactivat enteropathogens in sludges bein processed in rural and/or sma Publicly Owned Work Treatment (POTW).
3) Determine whether petroleum hydro carbons would inactivate enteropatho gens in non-hazardous petroleun sludges, petroleum-contaminated mu nicipal sludges, and pit muds bein co-disposed with municipal sludges.
4) Determine the appropriate controls fo studies of the survival of *Ascaris* egg

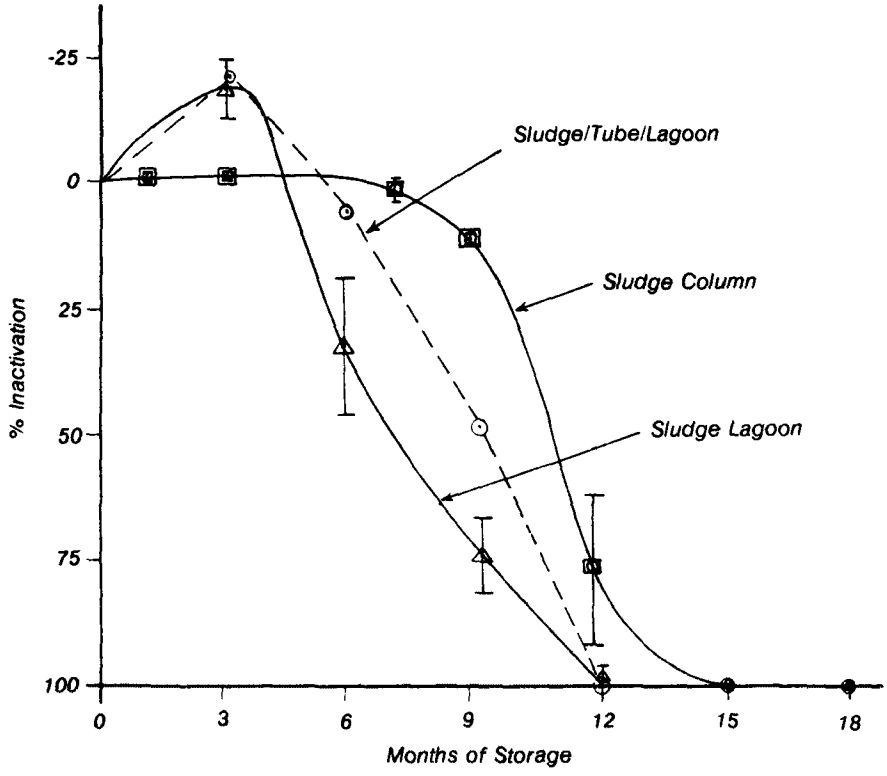

Figure 3. *Inactivation of* Ascaris *eggs stored in New Orleans sludge in lagoon, tube in the lagoon, and large column.*

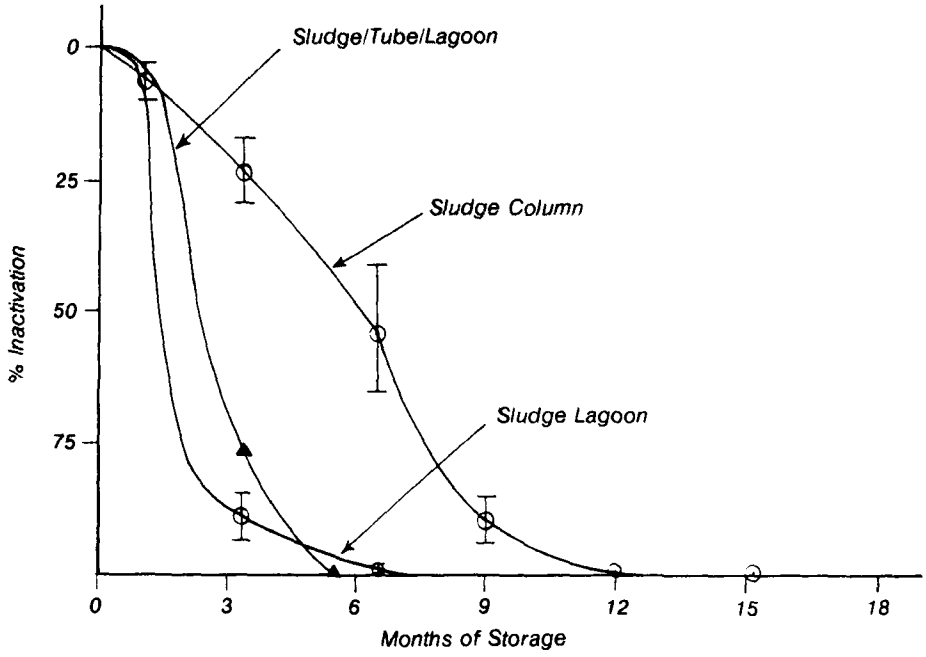

Figure 4. *Inactivation of* Ascaris *eggs stored in Port Aransas sludge in lagoon, tube in the lagoon, and large column.*

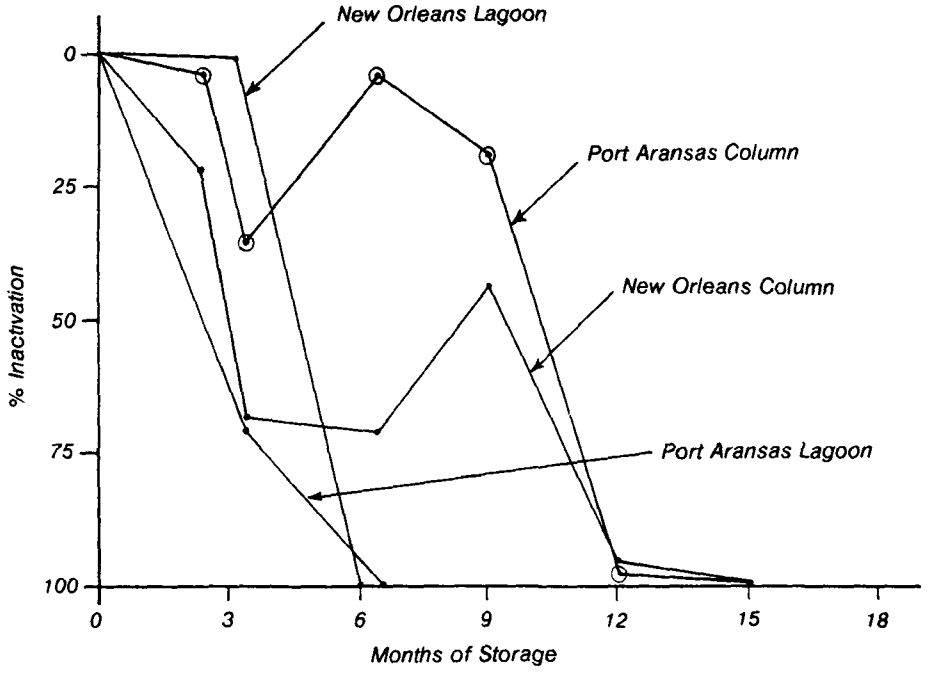

Figure 5. *Inactivation of Ascaris eggs stored in distilled water in vials stored in New Orleans and Port Aransas lagoons and large columns (controls).*

in different sludges or sludg products.

Conclusions

After 15 mo of storage in the Ne Orleans, Louisiana, and Port Aransa Texas, lagoons, *Ascaris suum* eggs we inactivated. Both localities are semitropical zones. In the Texas lagoc *Ascaris* inactivation was at a faster rat perhaps because of petroleu contaminants in the sludge. *Salmonel livingstone* and poliovirus Type 1 we inactivated within 6 mo of storage, ar total and fecal coliforms dropped 2 to logs. The fecal streptococci, howeve decreased very little.

With the New Orleans municip sludge, die-off of pathogens appeared be a result of temperature, where a *Ascaris* egg die-off in the Texa petroleum-contaminated sludge wa related more to petroleum residues th were estimated to be around 15% to 20% by volume.

Finally, the die-off data for both lagoc sites not only indicated pathoge reductions within 15 to 18 mo but was i accordance with published processes t further reduce pathogens (PFRP) i sludge treatment processes or proces schemes.

The full report was submitted i fulfillment of Cooperative Agreement Nc CR 810289 by Tulane University unde the sponsorship of the U.S Environmental Protection Agency.

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***Albert D. Venosa** is the EPA Project Officer (see below).*

The complete report, entitled "Persistence of Pathogens in Lagoon-Stored Sludge," (Order No. PB 89-190 359/AS; Cost: $28.95, subject to change) will be available only from:

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